STUDIES ON REGIONAL BLOOD FLOW IN THE KIDNEY AN APPLICATION OF THE THERMAL DIFFUSION METHOD

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An Application of the Thermal Diffusion Method

by

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Why should we be in such desperate haste to succeed and in such desperate enterprises? If a man does not keep pace with his companions, perhaps it is because he hears a different drummer. Let him step to the music which he hears, however measured or far away.

Henry David Thoreau

Walden, 1854

To the spirit of the Yale System and the libertarian tradition in Medicine which it sustains, this work is given.

Dr. Louis Levy's knowledge of the electronic equipment, philosophical conversations and carefully titrated support proved essential, while Mrs. Margaret Keaton provided a tolerant ally and a calming counterpoint.

Without my wife, Helle, whose Danish charm and understanding faith sustained me through long periods of doubt, this work could not have been completed.

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INTRODUCTION

The countercurrent theory of renal concentration of urine postulated by Wirtz, Hargitay and Kuhn (1) has focused attention on the regional distribution of blood flow in the kidney (2). The increasing importance of renal medullary hemodynamics in discussions of the urinary concentrating mechanism and the tubular reabsorption of sodium (3,4,5,6,33) and the difficulty of measuring or monitoring blood flow to this region of the kidney have led to a proliferation of indirect methods based on the clearance (Fick principle), dilution (Stuart principle), or diffusion of known quantities of easily measured substances delivered directly to the kidney circulation (6). None of these techniques, however, are suitable for the continuous monitoring of transient changes in regional distribution and rates of flow in the intact animal, nor are all of them applicable to all states of renal physiology, including oliguria. The need for a sensitive and direct monitoring device which does not interfere with the circulation being measured, has led to the development of flowmeters utilizing changes in the thermal diffusivity of tissues (7). Many workers have reported adaptations of this method to the study of specific tissues (8,9,10,11,12,13,14,29). This study presents its application to renal circulation.

The present method, utilizing a heated thermocouple flowmeter, measures the amount of heat input required to

maintain a constant thermal gradient across the thermocouple. It will be shown that the amount of this heat input, is proportional to blood flow. This technique is qualitative, but the amount of flow changes may be expressed as percentage of normal flow at any given site. In the medulla, the presence of urine flow as well as blood flow may affect the measurement of apparent thermal diffusivity. Recorded levels of medullary flow represent the effects of these two elements.

The major contribution of this technique is in the recording of continuing flow changes. Previous methods have demonstrated the distribution of total renal flow within the kidney and have given a static estimation of relative rates of blood flow within each hemodynamic region. They do not, however, yield information about either transient changes or more long term alterations in distribution or rates of blood flow in varying physiologic or pathologic states.

The heated thermocouple flowmeter used was a modification of the method of Levy et al.(8), which in turn represents a further refinement of the original technique of Gibbs (28) as modified by Grayson (22). Since the technique's inception repeated refinements, modifications, and technical improvements have been described (8,9,10,11,22). Its application to local tissue blood flow was originally demonstrated by Grayson (22), and its limitations defined by Linzell, Hensel, and others (9,10,12,13,30,31).

Within physiologic ranges of blood flow changes in apparent thermal diffusivity of living tissue is a function

of local blood flow when tissue temperature, arterial blood temperature and ambient temperature are constant.

For any system

1) Heat input (H) = I^2R

Where I = heating current &
R = resistence of heating wire

For any organic system

2) $H = \Delta H_T + \Delta H_B$

Where ΔH_T = heat added to tissue
and ΔH_B = heat added to blood

For tissue

2a) $\Delta T = \dfrac{\lambda \Delta H_T \sqrt{t}}{\sqrt{K \rho c}}$

Where λ = a constant
t = time
$\sqrt{K \rho c}$ = thermal diffusivity
since K = thermal conductivity
ρ = density
c = heat capacity

$$\therefore \Delta H_T = \frac{\Delta T \sqrt{K \rho c}}{\lambda \sqrt{t}}$$

For blood

2b) $\Delta H_B = Q(T_v - T_a)t$

Where Q = quantity of blood
T_v = temp.of venous blood
T_a = temp.of arterial blood

Substituting and combining 1 & 2

$$I^2R = \frac{\Delta T \sqrt{K \rho c}}{\lambda \sqrt{t}} + Q(T_v - T_a)t$$

Rearranging

$$Q = \frac{\Delta T \sqrt{K \rho c}}{\lambda \sqrt{t}\ (T_v - T_a)t} - \frac{I^2R}{(T_v - T_a)t}$$

In the present system ΔT is maintained constant by a servo system: at equilibrium $T_v - T_a$ is constant

$$\therefore Q = \frac{\text{Constant}}{\text{time}} - \frac{I^2R}{\text{time}}$$

Since R is also constant

Blood flow is proportional to heating current

A thermal gradient is established by heating one junction of a thermocouple. The heating current (I^2) is regulated by a servo system by means of which the heat gradient can be selected up to 2°C above ambient temperature. The probe and servo system are illustrated in Fig. 1. This method records the changes in I^2 and permits continuous monitoring of tissue blood flow.

When the heating current is off and there are no extraneous thermal gradients between the junctions, there are no observable changes in the differential thermocouple when blood flow characteristics are altered. This state of thermal equilibrium must be confirmed whenever flow changes are observed.

Recently Moffat and Fourman (15) reported microscopic anatomical studies of the renal vascular pattern of the albino rat as demonstrated by injection with Neoprene™ and dye. They distinguished four clear-cut zones of vasculature: 1) cortical, 2) subcortical, 3) outer and 4) inner medullary. They found an abrupt transition between the outer and inner medulla with the outer medulla distinguished by an extensive capillary plexus fed by branches from the vasa recta bundles,

while a less extensive capillary plexus is elongated in the inner medulla to form a loose meshwork between the straight limbs of the vasa recta. These are no longer in bundles and they terminate at various levels by breaking up into capillaries before forming ascending venous channels. The majority of vasa recta reach to the tip of the papillae where they and the capillaries are somewhat dilated. Other workers (16, 17, 18, 19, 20) have demonstrated that functionally the outer medulla and sub- or juxtamedullary cortex containing those glomeruli whose efferent capillaries supply the vasa recta may be considered a hemodynamically distinct compartment with flow rates greater than the inner medulla, yet considerably below those of the cortex as a whole.

It would appear from this work that flow in the outer and inner medulla is uniform in pattern while differing in amount (Fig. 2). The earlier studies of Trueta and coworkers (21) which led to the assumption of uninterrupted loops between descending and ascending limbs of the vasa recta within the inner medulla must therefore be modified. This is of importance, since the application of thermocouple flowmeters of the type used is related to the size of vessels, direction of flow and distance between probe and vessels. uninterrupted parallel flow would yield non-uniform areas of apparent thermal diffusivity which would have to be accounted for in interpreting results (7,12,22,30,31).

Methods used by other workers to study renal hemodynamics have included PAH clearance. On the basis of the

assumption that PAH in that fraction of blood perfusing the medulla is not extracted while that in the cortical fraction is completely extracted, the unextracted portion ($RPF-E_{PAH}$) has been considered to represent medullary blood flow (23). Thurau and his associates (6), however, have recently questioned the accuracy of this assumption. They believe instead that there is a net diffusion of PAH out of the collecting ducts and loops of Henle where PAH concentrations are high, into the medullary circulation, where it is low, and that part of the unextracted portion of PAH represents flow through the pelvic and hilar vessels. Thus, PAH extraction gives a falsely high measurement of medullary flow. The method is also unsuited to the oliguric state.

By applying the Fick principle to the diffusion of Kr^{85} Thorburn, et al.(16), have recently derived values for intrarenal nutrient flow rates from the decay curve of gamma emissions of the Kr^{85}. Analysis of these curves showed them to be describable by a series of exponentials, each representing the flow rate in different anatomical regions of the kidney. Four hemodynamically distinct regions of the kidney were thus differentiated and correlated anatomically with: 1) cortex, 2) juxtamedullary cortex and outer medulla, 3) inner medulla, and 4) perirenal and hilar fat. These functional regions correspond to the anatomical regions described by Moffat and Fourman (15).

The decay or washout curves of various other test substances have been employed to determine nutrient flow

rates within the kidney. These include dye (Evan's blue, Cardio green) (24), and radio-isotope labeled erythrocytes and plasma (Cr^{51}-tagged red cells and I^{131}-labeled albumin) (17).

All such applications of the Fick principle depend upon two assumptions: equilibration between blood and tissue of the test substance in one passage through the capillary bed, and low solubility of the test substance in blood relative to air with nearly total excretion via the lungs after one circulation and only negligible recirculation. There is evidence, however, that in the inner and outer medulla the removal of highly diffusible substances is affected by the countercurrent exchange diffusion between the ascending and descending limbs of both the vasa recta and loops of Henle (25). This tends to give calculated flow rates lower than the true values.

Several workers have estimated regional blood volumes by measuring the concentration of labeled albumin in quick-frozen slices taken from various regions of the kidney(18,19). From a series of slices taken from individual kidneys at different time intervals after the infusion of I^{131}-labeled albumin, Lilienfield et al.(20) were able to estimate a first approximation of plasma flow in the inner medulla. The diffusion of albumin into extravascular spaces and the higher concentration of protein in medullary vessels than in peripheral blood cause their calculated values of

intracapillary volume upon which flow is based to be too high (4,6).

Recently Kramer, et al.(6,26) have reported an ingenious application of the Stuart principle using mean circulation times derived from dye dilution curves recorded simultaneously by photoelectric probes placed on the cortex surface, aside the papilla and deeper within a calyx; the light for the latter probe being supplied by a miniature tungsten bulb at the tip of a hypodermic needle pierced through the tissue from above. This method has the advantage of yielding simultaneous data from the three hemodynamic regions of the kidney, but it does not escape the problem of recirculation of test material nor of insensitivity to transient changes.

Despite the diversity of methods employed and the theoretical and practical objections which have been raised, there is a surprising agreement as to the rate of the medullary blood flow among workers in this field (16,17,20,26,27). Thurau (6) recently summarized available data accumulated since Kramer's group (26) first demonstrated markedly reduced flow rates in the medulla as compared to the cortex using the dye dilution technique. Estimates of blood flow in ml./100 gms. tissue/min. in the cortex range from 450-475; for the outer medulla, 110-135, and for the inner medulla, 15-30. These values agree quite nicely with the calculated ideal inner medullary flow necessary to maintain increasing

concentration within the papilla consistent with the counter-current theory (3). Furthermore, the variability of these rates as found by various authors would help to explain changes in the glomerular filtration rate/tubular urinary flow balance (3,4,5). For the purpose of the present method a cortical/outer medullary flow ratio of 4:1 may be accepted.

PROCEDURE AND RESULTS

Cats were anesthetized with Nembutal™ and arterial and venous catheters placed in the femoral vessels. A trachael cannula was inserted through a tracheostomy. The kidney was exposed through a paraspinal incision and the thermocouple junctions sterotaxically placed on the surface of the cortex 1.5 cm. apart. The arterial blood pressure, output of the thermocouple and square of the heating current were recorded by a DC recorder. A constant thermal environment was provided and efforts made to maintain thermal equilibrium within the room.

The probes were stereotaxically advanced by 1 mm. increments through a nick in the renal capsule and levels of resting blood flow recorded at each successive depth. This procedure was repeated with the heat off. Typical results of depth exploration are shown in Fig. 3 and Graphs I and II.

At selected depths various vasoactive drugs were administered. These included Epinephrine, 0.02 mg.; Tensilon™ 1-4 mg., and Apresoline™ 5 mg. Each trial was repeated with the heat off. Fig's. 4 and 5 show typical results obtained for Epinephrine and Epinephrine and Tensilon™ after administration of Apresoline™, respectively.

The animal was terminated with an overdose of Nembutal™. The I^2 after death of the animal was recorded and resting levels of flow determined. After excision of the kidney the probe tract was marked with methylene blue

and the kidney fixed in formalin. The placement of the probe was ascertained by slicing the kidney and correlated with flow changes at each depth (Fig.6).

A thermal diffusivity curve was obtained for each probe (Graph III).

DISCUSSION

Measurement of blood flow by a heated thermocouple flowmeter is based in part on the assumption of an infinite and uniform sphere of tissue around the heated probe. It has been shown that the sphere of influence of a ΔT of 1°C has a radius of 5 mm. Furthermore, the apparent thermal diffusivity recorded will be influenced by several factors related to the geometry of the vasculature being studied, i.e., the size of the influencing vessels, spatial relationship of the probe to major vessels and direction of flow. In general, the most satisfactory results can be expected when the probe is placed in an area of uniform capillary and small vessel flow (12,22,31).

The application of this method to the study of flow changes in the kidney presents several problems. Firstly, the kidney of the cat is a relatively small organ, and the assumption of an infinite sphere of influence does not hold. The cortex, bound on one side by its capsule and on the other by the large calibre arcuate vessels, is relatively thin. This spatial limitation was partially compensated for by using ΔT's in the range of .4-.75°C. Secondly, the vasculature of the kidney is not uniform, and it is necessary to account for the influence of the large arcuate vessels located at the cortico-medullary junction. In the medulla apparent thermal diffusivity may be influenced by other factors than blood flow: a) urine flow and b) changes

in extravascular volume (12,31).

The influence of a large vessel on apparent thermal diffusivity has been shown to be describable by an asymtotic curve. Such a curve is shown in Graph II. The observed changes in I^2 at successive depths are probably in part a measure of the influence of high volume and rate of flow in the arcuate vessels. Graph I, plotting levels of I^2 recorded with the probes positioned so as to completely traverse the cortex, passing in contiguity with the arcuate vessels in the juxtamedullary cortex, before continuing on through to the opposite side, again reflects in part the relationship of apparent thermal diffusivity to distance from major vessels. If this factor were wholly responsible for observed changes in apparent thermal diffusivity, the magnitude of observed changes in I^2 with epinephrine infusion would be expected to show a similar relationship to proximity to the arcuate vessels, unless the response fell on the asymtotic portion of the curve. A consistent relationship, however, was not observed. The magnitude of epinephrine responses appeared to be constant at all depths greater than 3 mm. from the surface.

The response of flow to epinphrine in the medulla differed both in direction and duration from that observed in the cortex. The autoregulation of blood flow was clearly demonstrated in the cortex (34) while in the medulla no such response could be elicited. Thurau, et al. have reported

the absence of autoregulation in the inner medulla (32), and it is probably absent in the outer medulla as well (6). This characteristic difference is supporting evidence for a limited arcuate vessel flow influence, which should be uniform in all directions.

Systematic investigation of the influence of the arcuate vessels upon I^2 must await further refinements in experimental technique. Only gross stereotaxic and anatomical correlation of probe placement is presently possible. By using an experimental animal such as the dog, with larger kidneys, immobilizing the kidneys to eliminate shifts in the probe placement by respiratory and coughing movements, and using an inflexible probe, more accurate investigations may be carried out. The transpelvic route should make the medulla more accessible.

The effect of urine flow and changes in extravascular volume on apparent thermal diffusivity in the medulla have not been distinguished by the present work. It is probable, however, that the temporal relationship of urinary and vascular changes in flow with alterations in total renal blood flow is such that the observed rapid changes in apparent thermal diffusivity are primarily a function of changes in blood flow. Certainly the direction of flow changes in the medulla is not altered by urinary flow since epinephrine, which transiently decreases cortical flow and therefore GFR, produces a sustained increase in medullary flow. The

application of stop-flow and micropuncture techniques to the present method will be necessary to elucidate the exact relationship of these various factors to apparent thermal diffusivity.

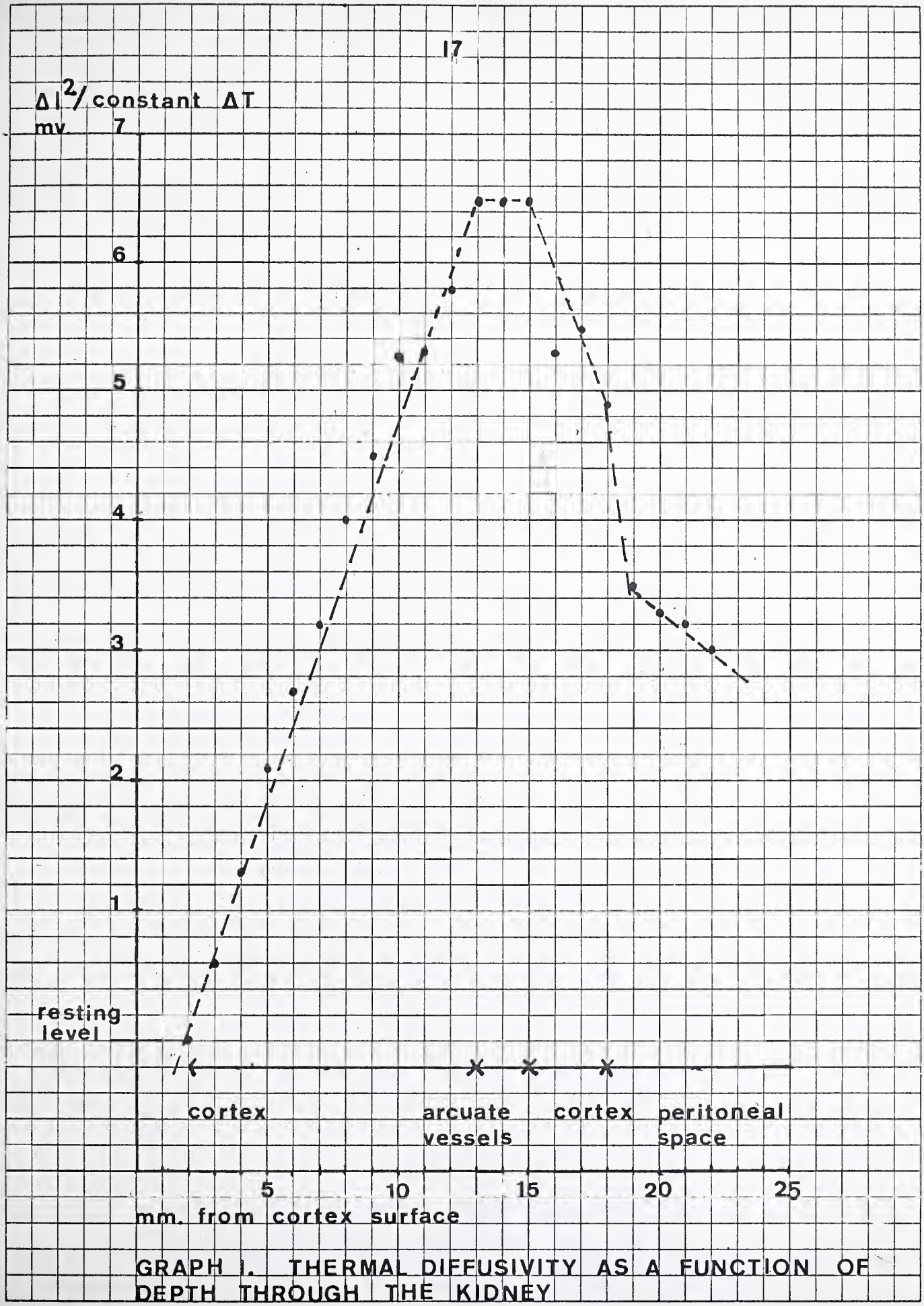
The pattern of cortical flow response to epinephrine was uniform throughout and could be observed at depths of 1 mm. from the surface. There was an abrupt, marked initial decrease of as much as 35% of resting flow with a prompt return to baseline (Fig. 4a). The time required for this return, between 5-10 sec., is in agreement with that observed by several workers for the autoregulation of renal blood flow (35,36,37,38). This pattern of response is significantly different from that recorded in the caudate nucleus of the brain by the same method (Fig. 7).

In the medulla blood flow responded to epinephrine with a somewhat delayed and more gradual increase which gradually diminished over 1-2 min. without exhibiting any autoregulatory response (Fig. 4b).

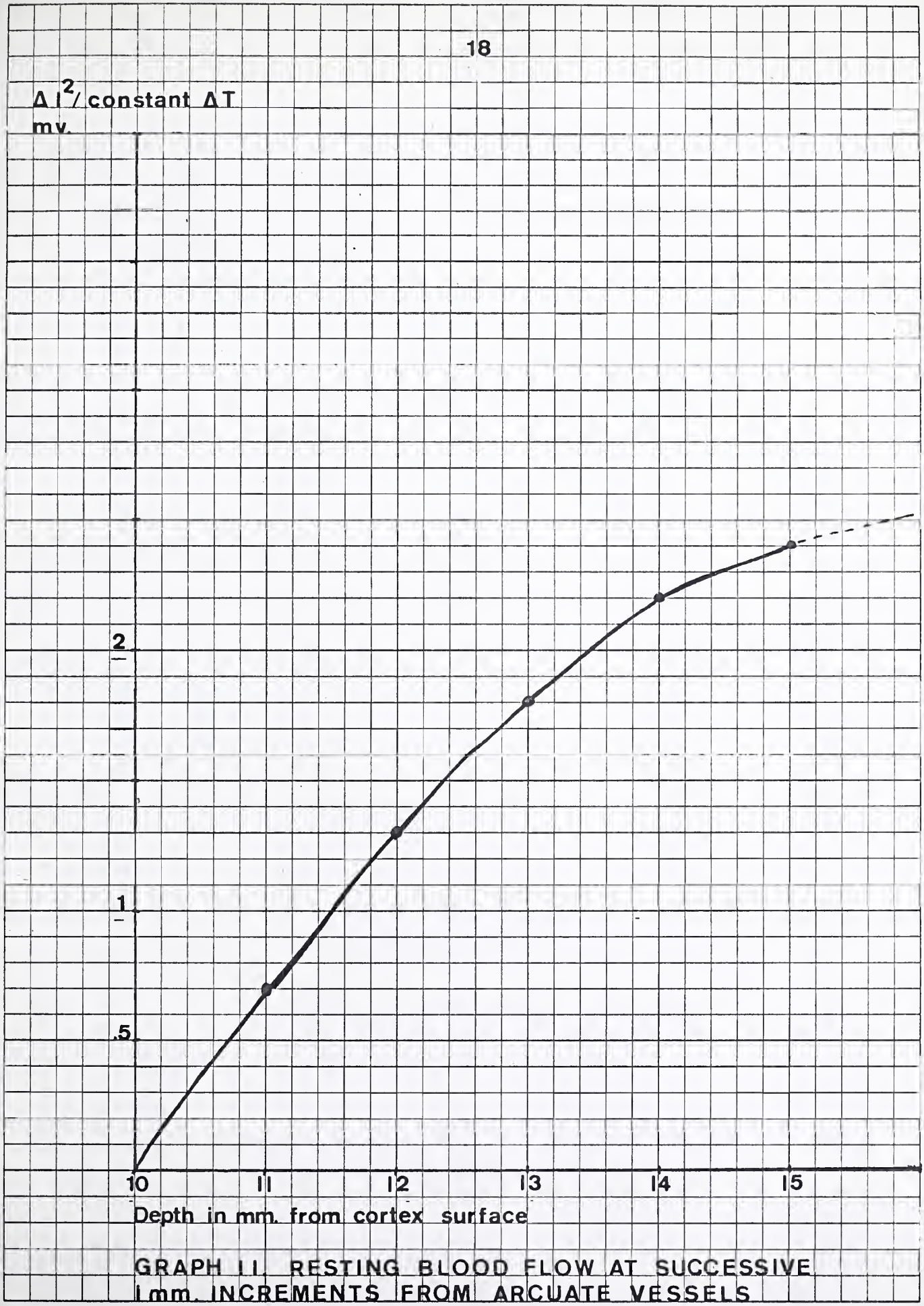
Fig. 5presents recordings of flow changes in the cortex with infusion of epinephrine and Tensilon™ before and after Apresoline™. No attempt was made to systematically investigate these responses; the data is presented to illustrate the versatility and sensitivity of the technique.

SUMMARY

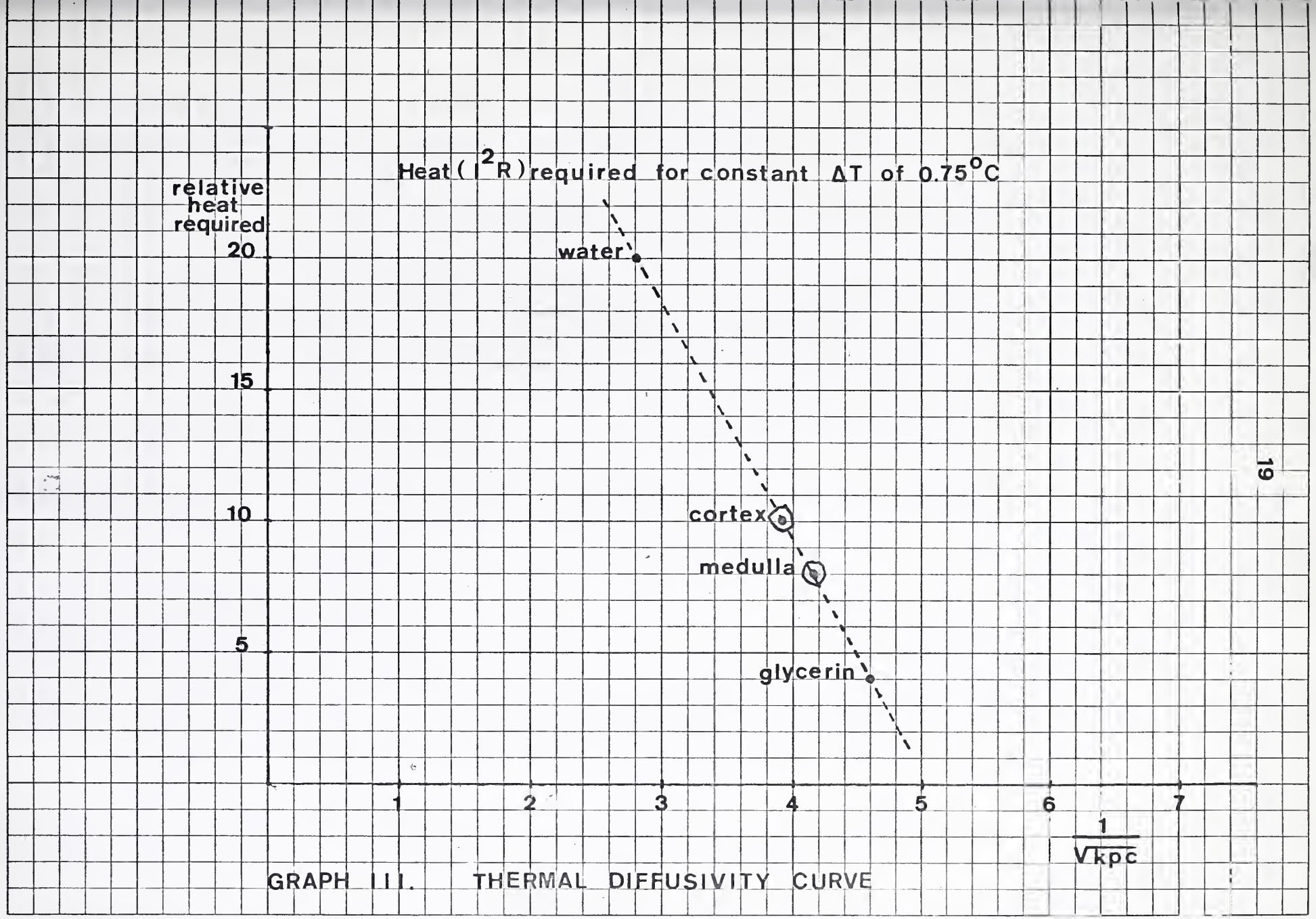
An adaptation of the heated thermocouple flowmeter method to the study of regional renal circulation is presented and its limitations explored. The pattern of blood flow changes after infusion of epinephrine was noted to differ in both direction and duration in the cortex as compared to the medulla. It is concluded that the heated thermocouple flowmeter provides a versatile and sensitive method for distinguishing and continuously monitoring transient blood flow changes in the cortex and medulla. Results suggesting a renal shunting of blood are presented.



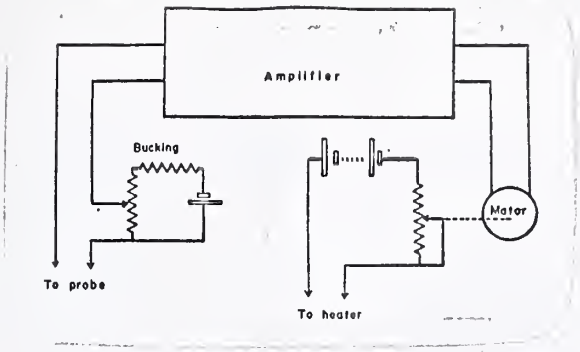
GRAPH I. THERMAL DIFFUSIVITY AS A FUNCTION OF DEPTH THROUGH THE KIDNEY



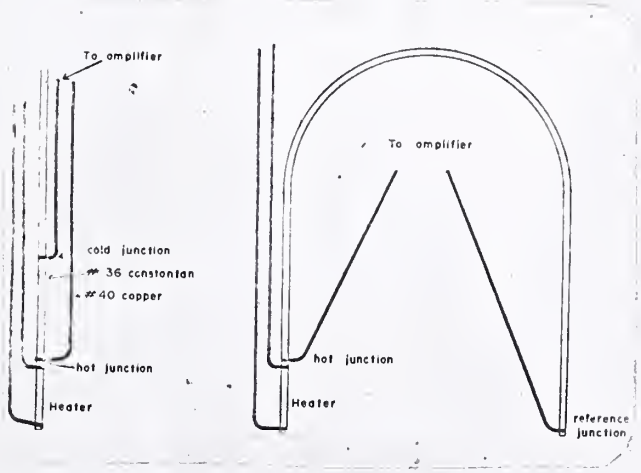
GRAPH II. RESTING BLOOD FLOW AT SUCCESSIVE 1mm. INCREMENTS FROM ARCUATE VESSELS



GRAPH III. THERMAL DIFFUSIVITY CURVE



a) Servo



b) Probe

Fig. 1 Servo & Probe

The vascular pattern of the rat kidney

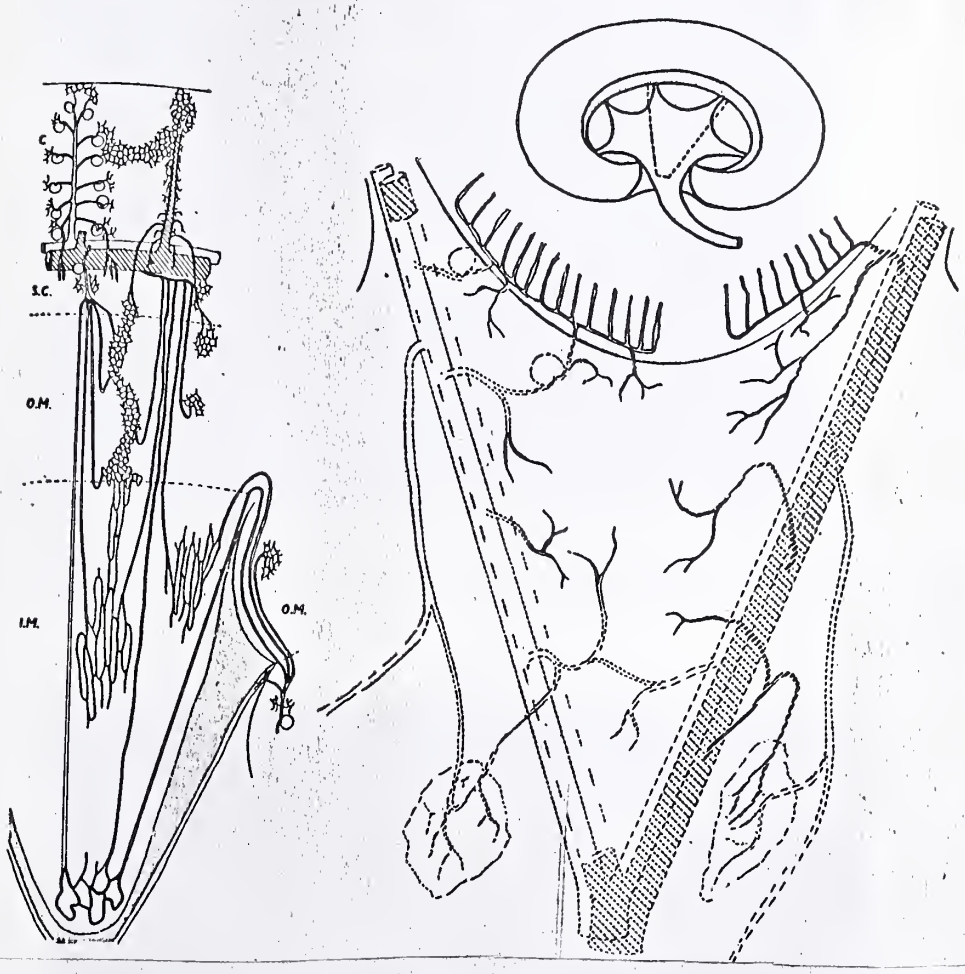


Fig. 2: From Moffat & Fourman (15)

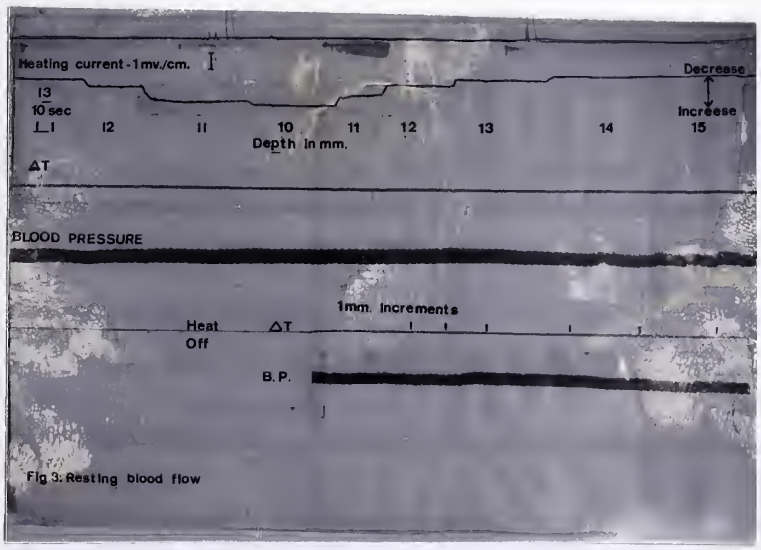


Fig 3. Resting blood flow

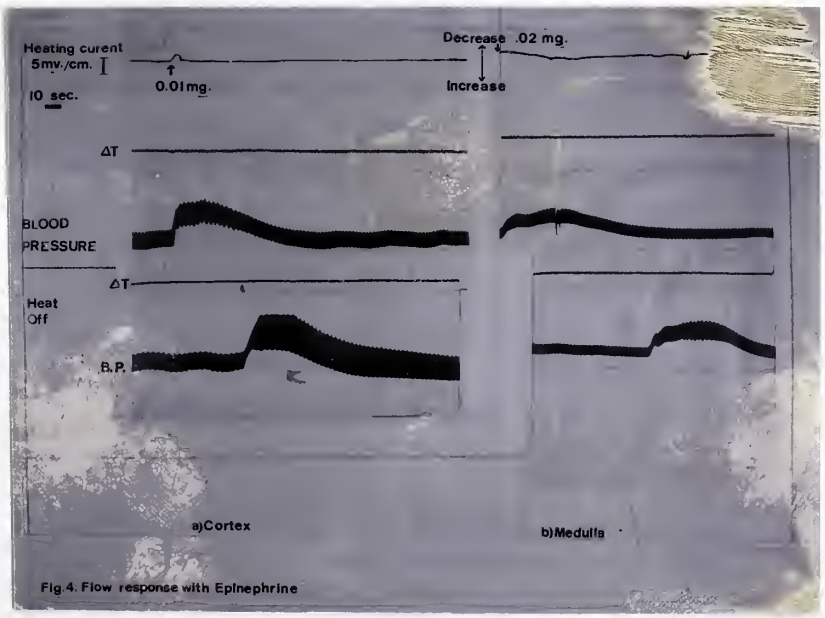


Fig 4. Flow response with Epinephrine

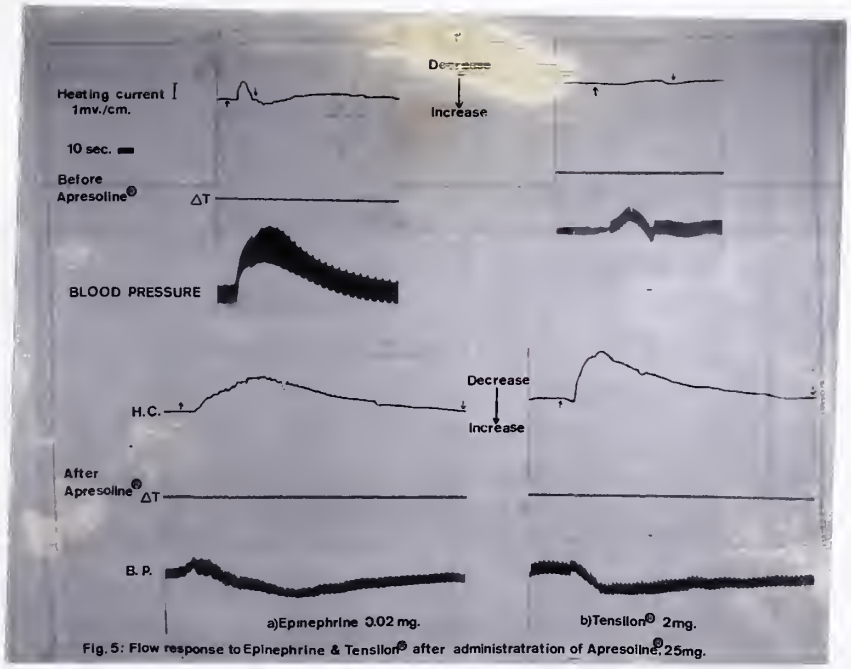


Fig. 5: Flow response to Epinephrine & Tensilon® after administratration of Apresoline® 25mg.

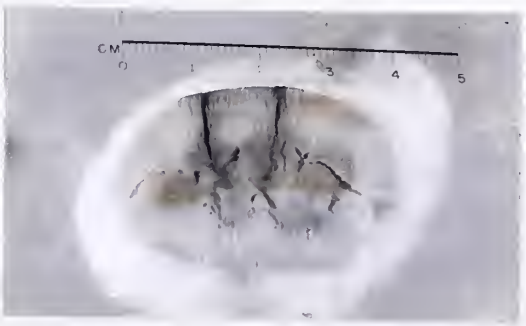


Fig. 6: Probe placement

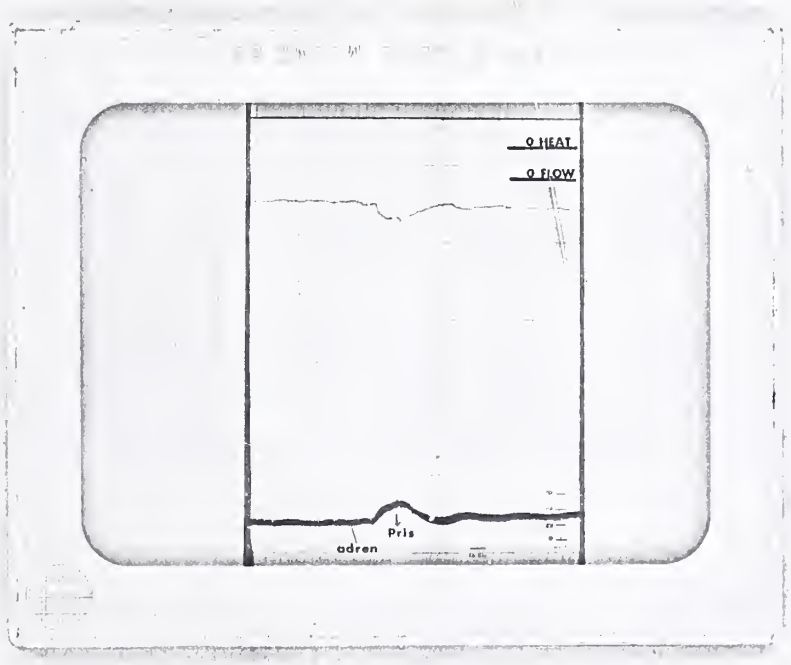


Fig. 7: Brain flow

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